Exhibit D

Current Knowledge and Research Priorities in the Digestive Manifestations of COVID-19

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As of May 10, 2020, approximately 4.2 million people worldwide have been diagnosed with coronavirus disease 2019 (COVID-19), and 280,000 have died, resulting in unthinkable human suffering and fundamental societal and economic disruption. While the responsible virus, severe acute respiratory syndrome novel coronavirus 2 (SARS-nCoV-2), has a primary affinity for the upper and lower respiratory tracts, the digestive system has emerged not only as a site of disease expression, but also as a possible driver of disease severity and viral transmission. Herein, we discuss the known digestive manifestations of COVID-19 and their potential implications, important questions that remain unanswered, and what gastroenterologists should know to care for affected patients and contribute to extinguishing the pandemic.

Current Understanding of COVID-19 and the Digestive System

Emerging evidence suggests that SARS-nCoV-2 infects the gastrointestinal (GI) tract. This is based on (1) a high incidence (in some reports) of digestive symptoms among infected patients,[1-4] (2) expression of angiotensin-converting enzyme 2 receptors—the viral target for cellular entry—throughout the digestive system,[1,2] (3) presence of viral RNA in the stool of infected patients,[1–3,5] and (4) prior experience with the 2003 SARS coronavirus and the 2012 Middle Eastern Respiratory Syndrome (MERS) coronavirus, both of which are known to infect and injure the GI tract.[2]

Reports from China have suggested that up to 50% of hospitalized patients and perhaps even more outpatients with COVID-19 will have at least 1 GI symptom, most frequently diarrhea or anorexia (Table 1).[2–4] Less common symptoms include abdominal pain, nausea, vomiting, dysgeusia, and bloody diarrhea. Importantly, studies suggest that a fraction of COVID-19 patients experience digestive manifestations in the absence of respiratory symptoms or fever—representing a potential source of exposure for unsuspecting healthcare workers.

Abnormal liver function tests (LFTs) have been reported in approximately 15%–50% of affected patients (Table 1).[6] The pattern of injury is largely hepatocellular. Most cases are mild (1–2 times the upper limit of normal) and self-limited, although severely elevated transaminases have been described and appear to correlate with the severity of systemic illness. A postmortem liver biopsy from a COVID-19 patient showed moderate microvesicular steatosis and mild lobular and portal activity, consistent with either direct viral infection or drug-induced liver injury; however, hepatocyte viral inclusions were not identified.[7]

Serum pancreatic enzyme elevations have also been noted in 9 of 52 (17%) COVID-19 patients in Wuhan, China.[8] Although no cases of severe pancreatitis occurred, the authors did observe acute elevations in serum glucose, potentially implicating the high level of angiotensin-converting enzyme 2 receptor expression in pancreatic islet cells.

Abbreviations used in this paper: **COVID-19, coronavirus disease 2019; GI, gastrointestinal; LFT, liver function test; MERS, Middle East respiratory syndrome; SARS-nCoV-2, severe acute respiratory syndrome novel coronavirus 2.**

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Table 1. Reported Digestive Manifestations of COVID-19

Digestive manifestation	Frequency reported (%)
Anorexia	30–40[3-4]
Diarrhea	2–50[2]
Nausea/vomiting	2–12[3-4,11]
Abdominal pain	2–4[3-4]
Digestive symptoms only	3–23[3-4]
Abnormal liver tests	
Aminotransferases	14–53[6,11]
Bilirubin	10–18
Virus detected in stool	50–55[1,3,5]

COVID-19, coronavirus disease 2019.

Multiple studies have confirmed the presence of SARS-nCoV-2 RNA in the stool of COVID-19 patients, including some who never tested positive in the upper respiratory tract. Additionally, the virus appears to persist in stool on average 11 days (and for up to a full month) after throat swabs become negative, and well after clinical recovery.[5]

Sound conceptual arguments but limited evidence implicate endoscopy as a possible hotspot for viral transmission.[9] Droplet contamination, based on bacterial culture swabs, has been demonstrated up to 6 feet away during colonoscopy. Additionally, viral aerosolization via peri- or intraprocedural coughing, retching, flatus, or even endoscope insertion and removal and accessory channel use is postulated. Based on these concepts and data from the SARS and MERS outbreaks, the American Gastroenterological Association has published guidelines recommending N95 (or equivalent) respirators for all upper and lower endoscopic procedures.[10] Additional recommendations include double gloving and the use of negative-pressure rooms for endoscopy in patients with known or suspected COVID-19. While earlier guidance favored a risk-stratified approach to using surgical masks vs N95 respirators, evolving knowledge of how the virus is transmitted and broad evidence of community spread now mandate maximal protection, including the universal use of hairnets, face shields, and shoe covers.

Advancing our Knowledge of COVID-19 and the Digestive System

While the recognition that SARS-nCoV-2 infection can present with isolated GI symptoms is crucial to reducing transmission, the overall importance of digestive manifestations in the natural history of COVID-19 remains uncertain. In contrast to the evidence presented previously, several high-profile series from China reported a very low incidence ($\leq$5%) of GI symptoms, perhaps owing to differences in case or symptom ascertainment.[11] Moreover, the severity of manifestations in varying reports has been inconsistent. Therefore, a more rigorous and systematic assessment of the prevalence, spectrum, and severity of digestive manifestations is necessary. To this end, the North American Alliance for the Study of Digestive Manifestations of COVID-19 (GI-COVID19.org) has launched a clinical registry aiming to better characterize the gastrointestinal and hepatic manifestations of COVID-19 across a network of approximately 30 centers in Canada and the United States.

Another important question is whether digestive symptoms in SARS-nCoV-2 infection are the result of direct injury to the GI tract (as was believed to be the case in 2003 SARS and MERS) or are simply a manifestation of viremia or systemic inflammation. Because the focus of care in affected patients is typically respiratory, and endoscopy is employed judiciously, direct evidence of gastrointestinal damage is largely lacking. While there has been a report of hemorrhagic colitis, the true association between SARs-nCoV-2 and epithelial damage remains unclear. As experience with endoscopic evaluation of COVID-19 patients grows, we should gain a more complete understanding of the endoscopic and histologic findings that might indicate viral injury. Similarly, an evolving understanding of hepatic manifestations in COVID-19 will elucidate whether abnormal LFTs reflect a direct viral effect (vs another competing etiology), whether their presence renders the patient more susceptible to drug-induced liver injury, and whether infection can precipitate decompensation of chronic liver disease, akin to hepatitis A.

If injury to the GI tract in fact occurs, then the presence of digestive manifestations may be associated with, and perhaps even contribute to, a more severe course of illness. Indeed, in limited reports, the magnitude of GI symptoms and LFT abnormalities in COVID-19 cases have mirrored disease severity. On this basis, it is important to clarify whether SARS-nCoV-2 induces a systemic inflammatory response through gut epithelial pathways. This seems plausible because modulation of lung injury by the gut microbiome and gut wall permeability—the so-called gut-lung axis—has been demonstrated in other contexts, including influenza infection.[12] Elucidating the possible connection between digestive manifestations and the overall severity of COVID-19 has important implications in prognostication and in developing GI-specific therapies that might impact disease severity.

The significance of SARS-nCoV-2 shedding in the stool also deserves more attention, because the presence of RNA does not necessarily indicate viral viability and infectivity. Beyond the need for heightened precautions in the handling of stool samples, cleaning of hospital rooms and endoscopy suites, and flushing of toilets, an understanding of the infectivity of stool has major implications in assessing the risk of food-borne illness—especially because one of the few remaining lifelines to our economy is take-out dining. Tracking food-borne infections in the midst of a pandemic illness primarily spread via aerosols from asymptomatic or presymptomatic carriers is challenging, although

putative mechanisms for fecal-oral transmission have been described, and at least 1 cluster of illness has been linked to buffet dining aboard a cruise ship.

In terms of endoscopy, universal precautions, as recommended by our professional societies, are essential. However, we do need more data on virus aerosolization during endoscopic procedures. This may have important implications in the re-expansion of endoscopy services and the reintroduction of trainees to the endoscopy suite. Some hospitals, for example, have instituted a mandatory 30- to 60-minute waiting period between cases (to allow dissipation of aerosols) and programmatic endotracheal intubation for all upper endoscopies, both reasonable practices during this crisis but not tenable in the long run. In addition, we need clarification on the performance characteristics, particularly the negative predictive value, of emerging point-of-care testing to risk stratify patients prior to elective endoscopy and to screen asymptomatic endoscopy staff. This, along with serological testing, and a clearer understanding of the immunity of recovered patients, may help elucidate a pathway toward relaunching fully operational endoscopy units, even in the face of future spikes or seasonality of COVID-19.

Many additional questions remain, including: does the virus lead to long-term damage to the gut and predispose patients to conditions such as post-infection irritable bowel syndrome and gastroparesis? What is the optimal evidence-based management of immunosuppressive medications patients with liver transplantation and inflammatory bowel disease? How will we re-expand endoscopy services to best serve our patients without putting them and ourselves at risk.

Take-Home Messages for the GI Community

In addition to embracing the widely publicized practices that aim to reduce transmission and flatten the curve—including the rapid adoption of telemedicine services—gastroenterologists should be doing the following as we await additional data.

First, educate their colleagues and healthcare systems to suspect COVID-19 in any patient with diarrhea—even in the absence of "typical" symptoms—and to take appropriate precautions.

Second, continue to approach endoscopy as a high-transmission-risk procedure by restricting it to nonelective cases and only when there is a high likelihood of therapeutic benefit in a known or suspected COVID-19 patient. Use (or reuse) an N95 respirator in every case and employ other personal protective equipment according to GI society guidelines.

Third, be involved in local and national discussions around the safe re-expansion of endoscopy services based on testing availability and accuracy and input from other important stakeholders.

Last, consider contributing to local or national research initiatives to answer some of the questions outlined previously. The North American Alliance for the Study of Digestive Manifestations of COVID-19 is seeking additional centers; the process for contributing is provided on GI-COVID19.org. Other registries evaluating COVID-19 in patients with prevalent inflammatory bowel disease and cirrhosis can be accessed at covidibd.org and covidcirrhosis.web.unc.edu, respectively.

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Conflicts of interest
The authors disclose no conflicts.